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Prostate Cancer (PCa) accounts for nearly 30% of all newly diagnosed cancers among American men. Epidemiologic studies suggest that dietary factors may be important in the etiology of PCa. The objective of our research is to determine how nutritional compounds genistein, betasitosterol (SIT), and omega-6 fatty acids (FA) function as modulators of PCa. In the second year of study, we finished preparation of all cDNAs for fabricating the custom microarray for analysis of gene expression. In addition, we completed in vitro and in vivo experiments using well established PCa cell lines and the three dietary compounds in varying concentrations. We are currently in the process of printing and validating the microarray and utilizing the array to detect gene expression patterns from the experiments. Finally, we have initiated xenograft in vivo experiments with a novel PCa model that was developed recently in a collaborating laboratory.

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Introduction

Prostate Cancer (PCa) accounts for nearly 30% of all newly diagnosed cancers among American men. Epidemiologic studies suggest that dietary factors may be important in the etiology of this disease. The objective of our research is to determine how nutritional compounds genistein, betasitosterol (SIT), and omega-6 fatty acids (FA) function as modulators of PCa. These three compounds belong to three distinct classes of dietary components, specifically isoflavonoid (genistein), phytosterol (SIT), and FA (omega-6 FA). Each class of compound could potentially modulate PCa in different ways. Previous investigations have indicated that all three compounds may affect PCa in a dose-dependent fashion. We will determine differences in gene expression profiles at a range of doses on *in vitro* models. We will then test two doses on *in vivo* models to account for the complexity of tumor microenvironments.

In the first year, we began to develop the technical tools with which to investigate gene expression patterns that are modulated by the 3 dietary compounds. In the second year of study, we finished preparation of 355 cDNAs for fabricating the custom microarray for analysis of gene expression. In addition, we completed *in vitro* and *in vivo* experiments using well established PCa cell lines and the three dietary compounds in varying concentrations. We are currently in the process of printing and validating the microarray and utilizing the array to detect gene expression patterns from the experiments. Finally, we have initiated xenograft *in vivo* experiments with a novel PCa model that was developed recently in a collaborating laboratory.

Body

Here, we report our progress as it relates to the approved Statement of Work:

Task 1: To delineate the gene expression patterns of PCa *in vitro* of three nutritional compounds.

1.1 Obtain cDNAs for microarrays

A total of 531 cDNA clones from either in-house resources or ATCC were tested for suitability for our custom microarray. Genes chosen were tyrosine kinases or were involved in cellular pathways with emphasis on androgen receptor (AR) and apoptosis pathways. Also included in our gene list are 3 control genes (GAPD, ACTB, and MBP). Of these 531 clones, 176 were discarded due to failure (i.e. lack of growth, not verifiable by PCR, sequencing and/or restriction enzyme digestion) leaving only 355 clones that are usable. Of the 355 usable clones, 65 have been sequence verified by ATCC. In addition, of the 355 usable clones, 323 contain sequences of unique genes while 32 are duplicates. All 355 clones have been purified and are ready for microarray printing.

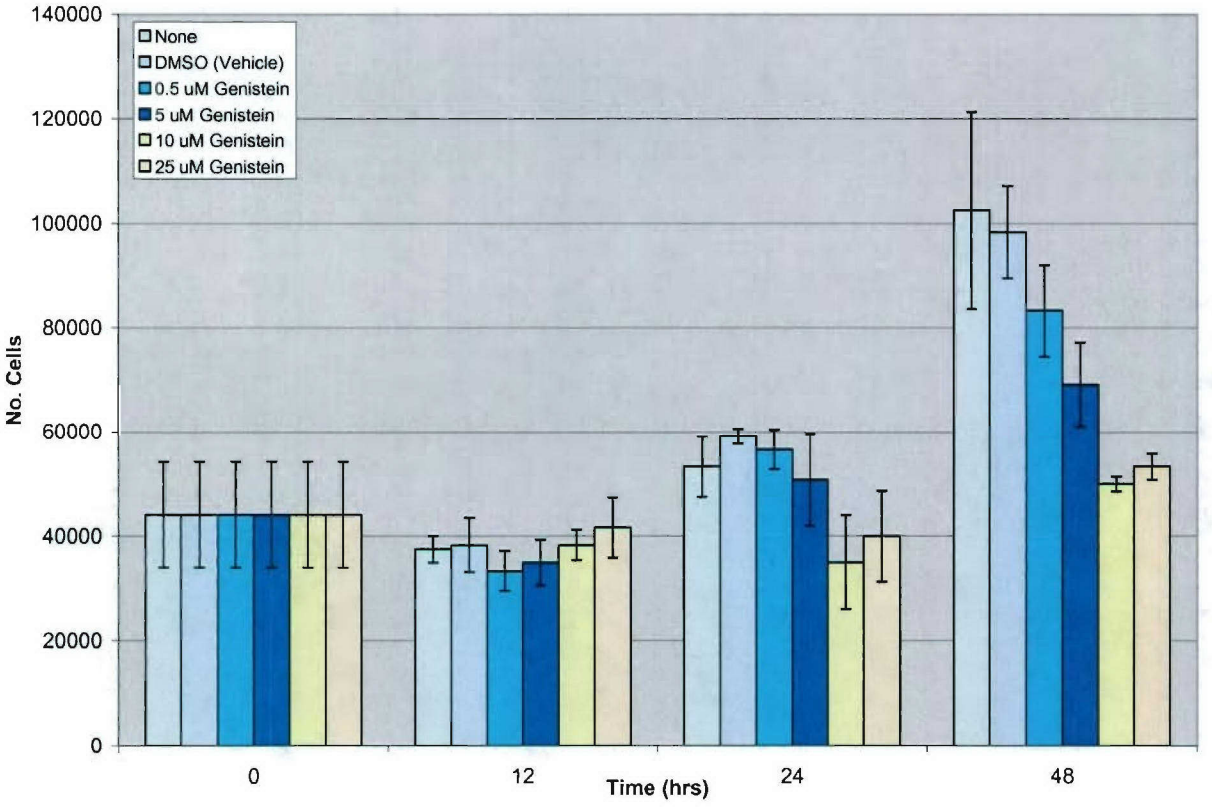
1.2 Fabricate arrays and optimize protocols

Due to delays in obtaining cDNA clones to complete our gene set for the custom microarray, we have only recently finished processing all usable clones but have not printed the DNAs onto the substrate. We anticipate that the fabrication, validation, and optimization of the custom array will be completed within the next 2 months.

1.3 Grow cells under test conditions, isolate RNA, RT-PCR, label probes

For this task, we have chosen to use well-known cell lines, PC3 and LNCaP, as well as three other PCa cell lines (BPH1 and its derivative lines CAFTD03 and CAFTD04) generated by the co-mentor. The cell lines were cultured in media containing four different concentrations of genistein, SIT, or omega-6 FA. Briefly, cells were seeded onto 6-well plates at a concentration of 5,000 cells per cm^2 surface area (or 48,000 cells) and allowed to attach for 12-18 hours in base media without supplementation with test compounds or vehicles. At time (t) = 0, the base media was replaced by base media, base media and vehicle (varies depending on test compound), or one of four test medias with different concentrations of test compound. Cells were counted at t = 0, 12, 24, and 48 hours after addition of test media with a change of media every 24 hours to ensure test compounds were readily available at the desired test concentration. Each experiment was done in triplicate and the average of the three experiments is used for comparison. An example of growth patterns obtained by this *in vitro* system for CAFTD04 is shown in Figure 1.

Figure 1: CAFTD04 cells cultured in different concentrations of Genistein over 48 hours. Each data point reflects the average of data obtained from triplicate experiments and error bars reflect one standard deviation.



1.4 Perform microarray experiments, analyze data with standard statistical programs and Perform multicolor spectral transcript analysis and analyze data with standard statistical programs

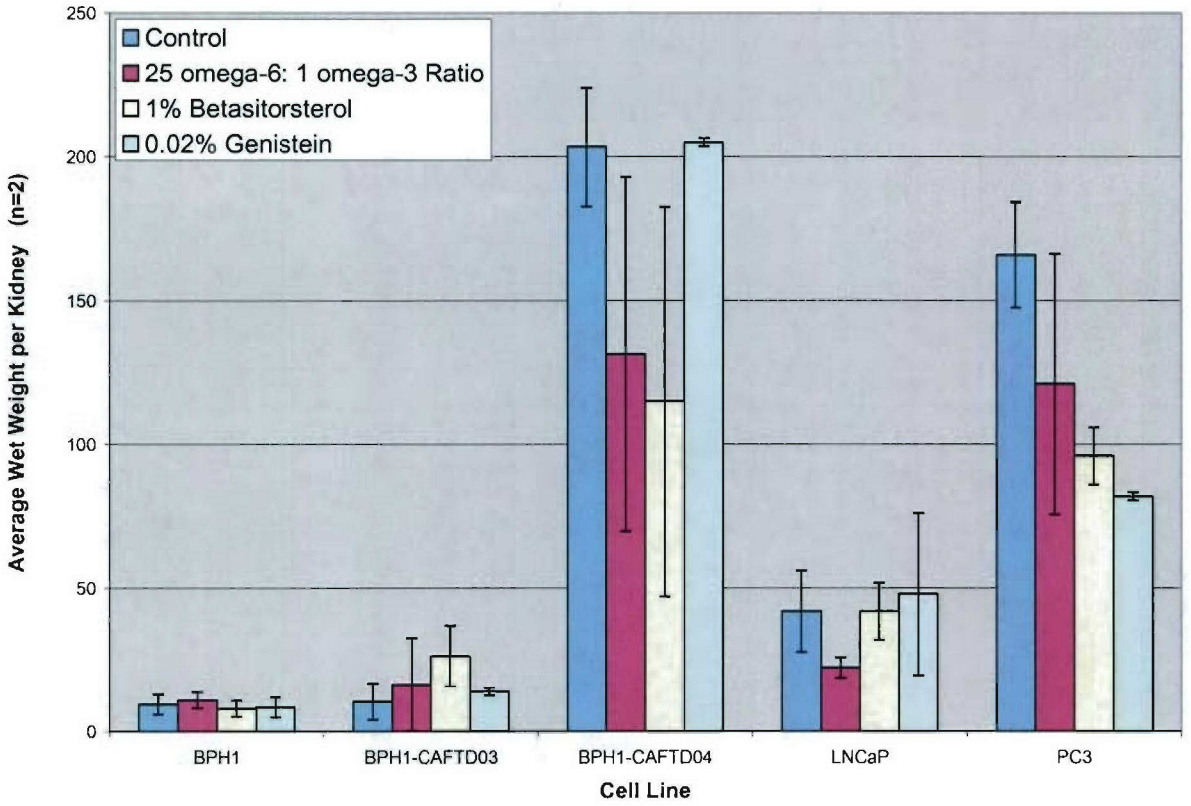
Due to the delay in the custom microarray fabrication, we have not been able to analyze expression patterns from RNA isolated from the *in vitro* experiments. We anticipate performing this task in the next 6 months.

Task 2: To determine if and how three select dietary components modulate the growth and gene expression of clinical PCa specimens in vivo.

2.1 Pilot study to investigate timing of nutrient supplementation in SCID mice

In parallel with the *in vitro* experiments mentioned in Task 1, we have performed *in vivo* experiments using xenograft technique on SCID mice at the co-mentor's laboratory using the same cell lines mentioned above. Briefly, cells are implanted into the kidney capsule of SCID mice and allowed to grow for 21 days. Starting from the day of the xenograft surgery, we began feeding the mice with control (no additional Genistein or SIT and omega FA ratio of 48 omega-6: 1 omega-3), FA ratio of 25 omega-6: 1 omega-3, 1% SIT, or 0.02% Genistein diets. This was done to determine if the test compounds will affect the implantation of the tumor cells. In addition, omega FA ratios of omega-6: omega-3 for the FA test diet needed to be reduced (as opposed to increased as originally planned) due to the high omega-6: omega-3 ratio of the control diet (as in most rodent diets). The planned increase of omega-6 content may be a cause for concern for the animal. Only one test diet per compound was chosen due to cost and nature of this pilot study. At day 21, tumors were harvested and frozen for subsequent microarray and other analyses. Each experiment was done in duplicate and the average of the data from the 2 experiments is plotted on Figure 2.

Figure 2: Affect of different test diets on different PCa cell lines in vivo.



2.2 Analyze tumors from pilot study

Similar to task 1.4, we have not been able to complete this task due to delays in microarray fabrication.

2.3 Grow 6 tumors in 21 SCID mice (2 tumors per mice) that ingest one of 7 test diets, harvest tissues, prepare tissue sections and RNAs, RT-PCR, and make probes. Test diets include control and each compound at one of 2 concentration

For this task, we have found it difficult to obtain clinical samples that were large enough to be implanted into the number of mice that was initially proposed. To circumvent this problem, we are looking to a newly generated PCa model that was developed by a collaborator, (Wang et al., submitted). Briefly, pieces of PCa tissue from a patient were grafted into the subrenal capsule site of testosterone-supplemented male SCID mice. After 5 serial transplantations, the tissues were transferred into mouse prostates. A metastatic tumor line generated from lymph nodes, designated PCa1-met, had few chromosomal alterations, as indicated by Spectral Karyotyping. Orthotopic grafting of PCa1-met in 47 hosts led in all cases to metastases to multiple organs (lymph nodes, lung, liver, kidney, spleen and, notably, bone). Histopathological analysis showed strong similarity between orthotopic grafts and their metastases which were of human origin as indicated by immunostaining using antibodies against human mitochondria, androgen receptor, prostate-specific antigen, pan-cytokeratin, p63, cytokeratin-8 and Ki-67. This model is unique due to the fact that the xenograft implants metastasized to regions of the mouse that paralleled the locations of clinical PCa metastases (most notably lung, liver and bone) in humans. In addition, the resulting cell lines contain few cytogenetic aberrations, similar to clinical PCa and unlike most well established cell lines (i.e., PC3, LNCaP, and DU145). We are currently testing 6 cell lines derived from the original clinical samples for their suitability for this study. The benefits of the results from these experiments are:

1. Human PCa origin
2. Availability of samples
3. Multiple samples from the same patient
4. Metastatic lesions (to sites that resemble clinical cases) for optional further analysis
5. Similarity to clinical samples (in terms of number of cytogenetic aberrations)

2.4 Perform microarray experiments, analyze data with standard statistical programs

We are anticipating task 2.3 will be completed within 3-6 months, which will provide the samples necessary for this task in subsequent months.

Key Research Accomplishments for Year 2

- Prepared 355 cDNA clones consisting of tyrosine kinase genes as well as other genes in various cellular pathways with emphasis on androgen receptor and apoptosis pathways.
- Performed *in vitro* experiments with established PCa cell lines grown in control and test media containing different concentrations of test nutritional supplements
- Performed *in vivo* experiments in the co-mentor's laboratory with xenografts of PCa cell lines in SCID mice whose diets are supplemented with various test compounds.
- Initiated in vivo experiments with newly developed PCa models in co-mentor's laboratory

Reportable Outcomes

- Obtained National Cancer Institute Cancer Prevention Fellowship for 2005-2008
    - Beginning in July 2005, the fellow will begin a 1 year academic program to obtain a Masters in Public Health with an emphasis on Epidemiology at the University of California, Berkeley to supplement the fellow's training in the field of cancer prevention
    - Beginning in June 2006, the fellow will begin a 2 year research program at the National Cancer Institute in Bethesda, MD, which will focus on cancer prevention.

Conclusion

We have used the second year of this fellowship for finishing preparation of cDNAs for our custom microarray fabrication. The custom array will have 355 features including tyrosine kinase genes, genes involved in androgen receptor and apoptosis pathways among others. In addition, the fellow has finished *in vitro* and pilot *in vivo* experiments necessary to generate RNAs for expression analysis. Furthermore, a new model of PCa progression has been obtained for the purpose of new in vivo experiments to help complete task 2. We have also requested and been granted a one-year no-cost-extension to complete this project.

Publications

Weier JF, Weier HU, Jung CJ, Gormley M, Zhou Y, **Chu LW**, Genbacev O, Wright AA, Fisher SJ. Human cytotrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype. Developmental Biology. 2005 Mar 15;279(2):420-32.

Chu LW and Liang JC 2005. "Detection of Genetic Abnormalities Using Comparative Genomic Hybridization in Prostate Cancer Cell Lines" in *Handbook of Immunohistochemistry and in Situ Hybridization of Human Carcinomas : Molecular Pathology, Colorectal Carcinoma, and Prostate Carcinoma*, edited by M.A. Hayat, 327-334. London, San Diego, Burlington: Elsevier Academic Press.